

Morphology and ultrastructure of isolated gemmae of *Drosera pygmaea* and their *in vitro* germination

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Abstract

Gemma morphology, histology and ultrastructure before and after germination *in vitro* were studied in *Drosera pygmaea*. The histology of the gemma is similar to that of a seed, being characterized by an embryo-like structure and storage tissue, although no seed coat is formed. One embryo-like structure within the gemma, which gives origin to a new plant, expresses polar organisation with distinct meristematic regions. Storage tissue surrounding the embryo-like structure resembles endosperm and it is built of parenchyma cells possessing plastids with starch grains and dense material within vacuoles. The regeneration from the gemma may provide useful system to study plant morphogenesis under stress conditions including *in vitro* culture.

Additional key words: histology, scanning electron microscopy.

Introduction

Several plant species like ferns and lichens can produce special vegetative reproductive organs and tissues. Gemma formation in *Drosera pygmaea* represents a special type of vegetative reproduction during which specialized reproductive organs - stipule bud and gemma are developed as a natural alternative to flower and seed production. Gemmae reproductively play the same function as seeds, but without any

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fertilization process and fusion of gametes. By contrast to sexual reproduction from haploid gametes, gemmae originate from diploid somatic plant cells.

Drosera species are usually reproduced by seeds. However, gemma differentiation may be induced as an efficient alternative system for plant reproduction under changing environmental conditions as a response to reducing photoperiod and lower temperature. This type of vegetative reproduction was briefly described by Slack (1980) and Lowrie (1989).

No comprehensive information concerning gemma morphology, anatomy and ultrastructure has been reported previously. In this work we provide first original data in this field showing some cytological aspects of isolated gemmae including their germination *in vitro*. We have also aimed to develop an effective *in vitro* multiplication system for *Drosera pygmaea*.

Materials and methods

Gemmae were isolated from stipule buds (using forceps under a binocular microscope) which were formed on mature *Drosera pygmaea* plants *in vivo* or *in vitro*. They were surface sterilized by 70 % ethanol for 30 s and subsequently in 2.5 % solution of *Chloramin B* (analogous to calcium hypochloride). Gemmae were cultured *in vitro* and germination was achieved on MS medium (Murashige and Skoog 1962) without growth regulators, using temperature of 25 ± 2 °C, 16-h photoperiod, and irradiance of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ (300-700 nm).

After 0, 3, 7, 9 and 11 d in culture, samples were fixed in 3 % glutaraldehyde for 5 h and postfixed in 1 % OsO_4 for 2 h. After dehydration in a graded acetone series they were embedded in *Durcupan ACM* resin (*Fluka*, Buchs, Switzerland).

Semithin sections (1 μm thick) for light microscopy were cut on an *Reichert OM U3* ultramicrotome (*Reichert*, Vienna, Austria) and double stained with toluidine blue and basic fuchsin. Ultrathin sections (80 - 110 nm thick) for transmission electron microscopy were prepared using the same ultramicrotome, stained with uranyl acetate and lead citrate and examined in a *JEOL 2000 FX* electron microscope (*JEOL*, Tokyo, Japan).

Samples for scanning electron microscopy were fixed as described above, dehydrated in ethanol and dried using critical point dryer *CPD 020* (*Balzers*, Vaduz, Liechtenstein) with liquid CO_2 . They were stuck with silver paper to metal blocks, covered with colloidal gold using *JEOL* sputter coater and observed in a *JEOL JXA 840B* electron microscope.

Results

Gemmae are differentiated on the basal part of the stipule bud in mature plant of *Drosera pygmaea* under changing environmental conditions (reducing in photoperiod, lower temperature) *in vivo* (Fig. 1). Their complete development takes about 30 - 40 d. It was possible also to induce stipule bud formation and gemma

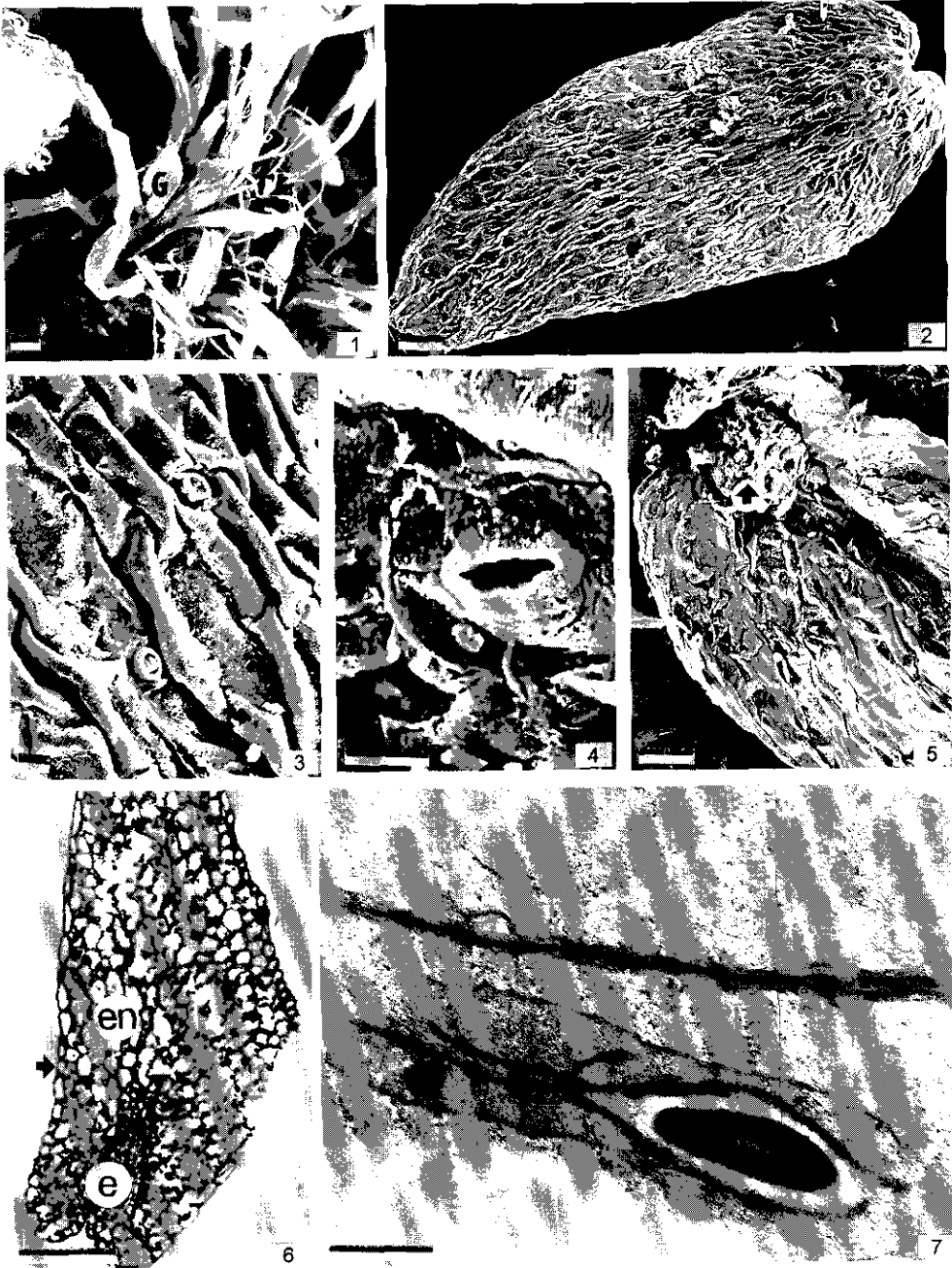


Fig. 1. Gemma (G) on the basal part of a stipule bud in a mature plant of *Drosera pygmaea*. Bar = 100 μ m.

Fig. 2. Overview of a mature gemma. Bar = 100 μ m.

Fig. 3. Detail of gemma surface. Bar = 10 μ m.

Fig. 4. Anomocytic type of stomata - detailed view. Bar = 10 μ m.

Fig. 5. Globular embryo-like structure (arrow) located on the basal part of gemma. Bar = 100 μ m.

Fig. 6. Semithin section through a gemma. Embryo-like structure (e) is surrounded by endosperm-like tissue (en). Epidermis is indicated by arrows. Bar = 100 μ m.

Fig. 7. Chloroamyloplast and mitochondria in gemma cells. Bar = 5 μ m.

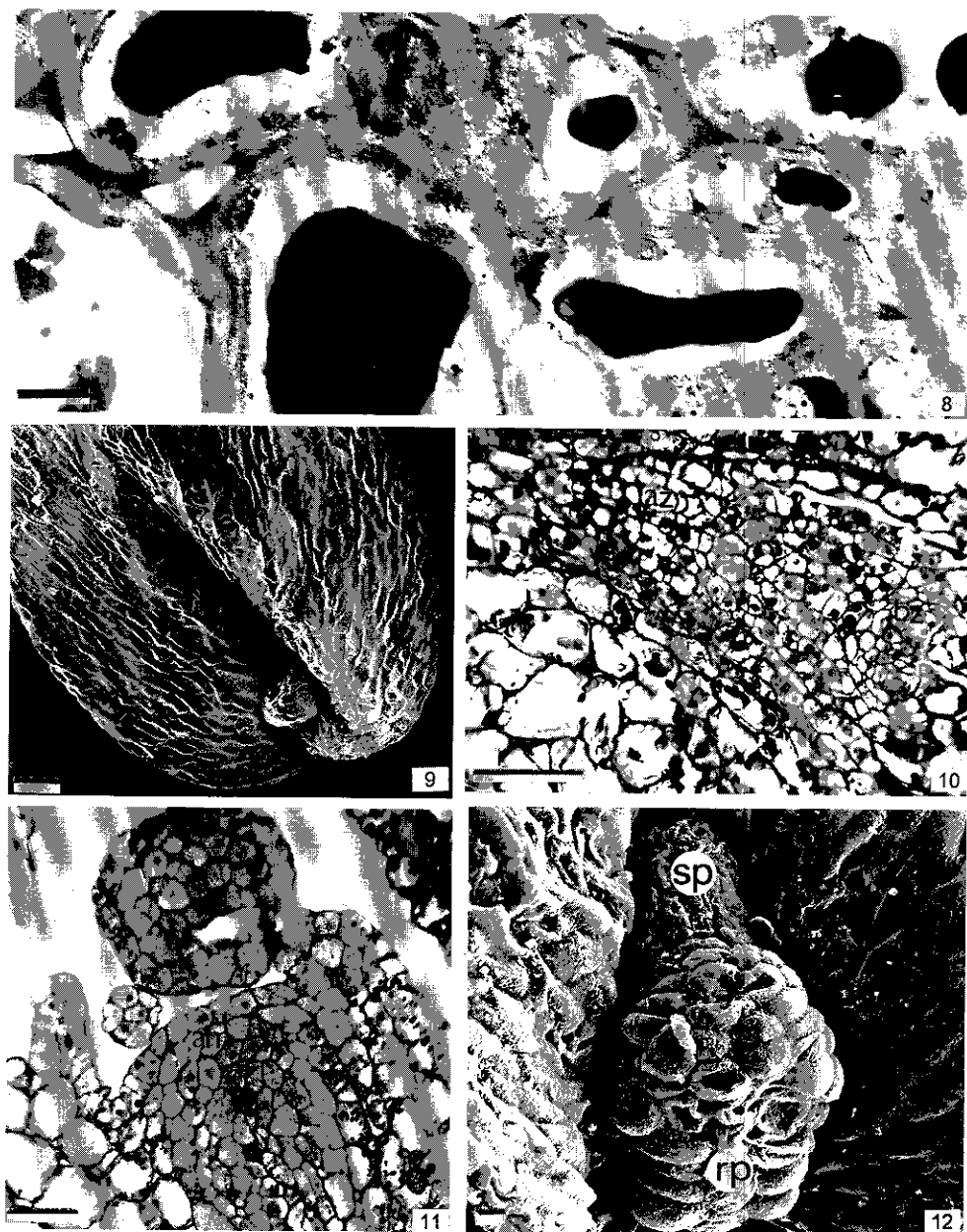


Fig. 8. Aggregation of large dense bodies in the central vacuoles of the endosperm-like cells. Bar = 10 μ m.

Fig. 9. SEM view of the pear-shaped embryo-like structure (e) after 3 d in culture. Bar = 100 μ m.

Fig. 10. Histological view of gemma - apical zone (az) and basal zone (bz). Bar = 50 μ m.

Fig. 11. Shoot apical meristem (am) with leaf primordia on its sides. Bar = 50 μ m.

Fig. 12. Differentiated shoot (sp) and root poles (rp) as viewed by SEM. Bar = 10 μ m.

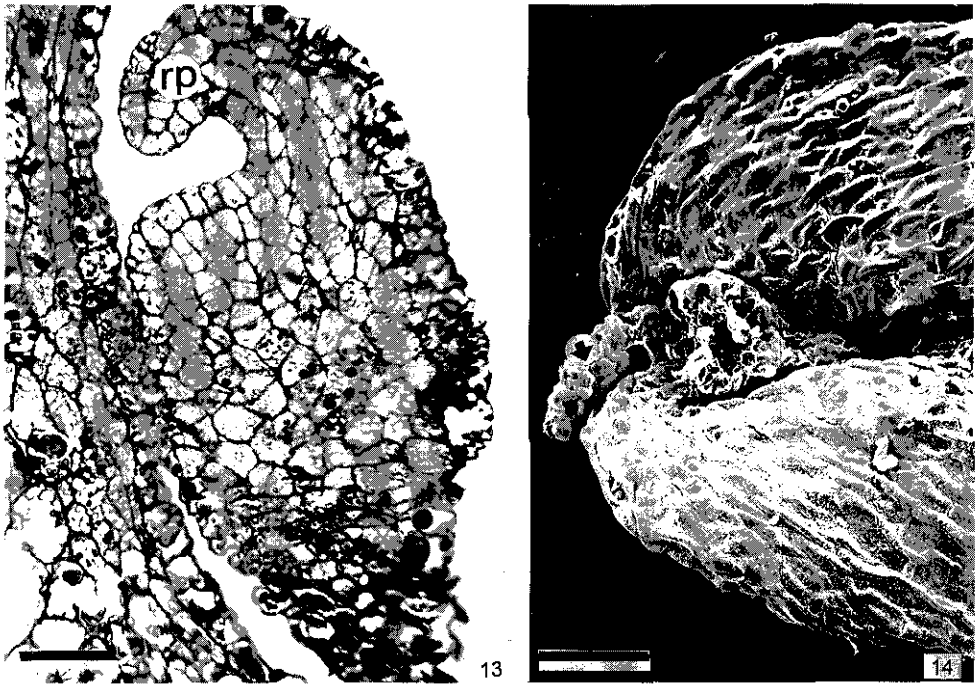


Fig. 13. Section through an embryo-like structure after 7 d of germination *in vitro* (rp - root primordium). Bar = 50 μ m.

Fig. 14. Developed primary root (arrow) after 11 d in culture. Bar = 100 μ m.

production *in vitro* using MS culture medium without growth regulators. The mature gemma has an oblong shape. It is about 1 mm long, 0.6 mm wide and 0.5 mm thick. The apical part is smooth. The basal part of the gemma which is in contact with the leaf petiole has a heart-like shape (Fig. 2). Each gemma is covered by an epidermis with numerous stomata located especially in the apical and central part of the dorsal side (Fig. 3). Stomata are of the anomocytic type (for an open stoma see Fig. 4).

Using SEM method it was shown that globular embryo-like structures are located to the basal part of the gemma ventral side, and they are surrounded by storage tissue (Fig. 5). Embryo-like structure has an apparent polar organization and is composed of parenchymatic and meristematic cells located within meristematic regions (Figs. 6, 10). These polarly-located meristems give an origin to the shoot and root primordia during *in vitro* gemma germination. Each embryo-like structure is surrounded by storage tissue resembling the endosperm of seed plants and is composed of round-shaped and oblong cells. These are parenchymatic having a big central vacuole and peripheral cytoplasm which contains numerous plastids with starch grains (Figs. 6, 10). Each gemma is covered by one layer of tightly arranged cells representing an epidermis. Only the outer periclinal cell walls of epidermis are thickened.

The differentiation of shoot primordia from the originally pear-shaped embryo-like structure can be observed during gemma germination *in vitro* after 3 d in culture. The shoot apex is relatively wide. Division activity of laterally located cells within apex leads to the formation of lateral meristematic zones from which leaf primordia arise (Fig. 11). *Organ primordia (shoot and root poles) are differentiated within the germinating embryo-like part of gemma after 7 d in culture (Figs. 12 and 13). One layer of epidermal cells is formed on the shoot primordium surface covering also the apical meristem.*

Primary root is developed after 11 d in culture (Fig. 14). Complete new plants were regenerated from germinating gemmae *in vitro* after 6 weeks. They were grown to maturity and transferred to the soil.

In endosperm-like tissue, the ultrastructural features are typical of parenchymatic storage cells. Plastids are represented by chloroamyloplasts having poorly developed lamellar system. They possess large starch grains and several plastoglobuli within their stroma (Fig. 7). Mitochondria are not numerous and have only several cristae. Almost two thirds of storage cells are occupied by a large central vacuole which is regularly filled with dense bodies (Fig. 8).

Discussion

Previously, Lowrie (1989) reported that gemmae consist of nutritive tissue and embryogenic leaf and root structures, however, no detailed anatomical and morphological studies were performed. Except this brief mention there is no other information about gemma structure. Nothing is known about gemma origin and development. Albert *et al.* (1992) proposed that appearance of gemmae in place of trapping lamina is homeotic and likely represents a shift in cell differentiation rather than development of *de novo* structures. Here we provide for the first time an evidence, that mature gemmae are anatomically and functionally similar to the seeds. They possess embryo-like structures with polarly organized meristems and endosperm-like storage tissue.

Our data indicate that MS medium without growth regulators is suitable for the micropropagation and morphogenesis of *Drosera pygmaea*. *In vitro* conditions, moreover, can promote gemma production in this species by simulating natural stress conditions, and the gemmae can then be used like explants during *in vitro* propagation. For example, Karlson and Pate (1992) reported increased gemma production in several sundew species (8 - 52 gemma per plant during the season) compared to the less-productive seed formation (just 8 mature seeds per plant during the season), suggesting that this vegetative type of reproduction is more effective. Plants in their natural environmental conditions create gemmae instead of seeds, because it takes less time after a dramatic change of environment. It can be considered as a functional adaptation to stress. Gemma germinate efficiently (80 - 100 %) for up to 7 - 14 d since maturity. Plant development from the gemma to a mature plant takes only 70 - 90 d compared to 120 - 150 d from mature seed (Lowrie 1989). Here we show that gemmae are able effectively germinate during

in vitro culture and they represent useful source of explants for micropropagation of *Drosera pygmaea*. Previously, the plant regeneration *in vitro* was described in two *Drosera* species - *Drosera rotundifolia* and *Drosera spathulata*, and perspectives for the efficient micropropagation of these species were discussed (Simola 1978, Beebe 1980, Bonnet *et al.* 1984, Crouch *et al.* 1990, Blehová *et al.* 1990, 1992, Bobák *et al.* 1993). In our recent work, we reported about an efficient regeneration system from isolated leaves of *Drosera rotundifolia* (Bobák *et al.* 1995). Interestingly, Anthony (1992) claimed that *Drosera rotundifolia* and *Drosera capensis* are not only able to grow *in vitro*, but also give flowers and seeds when cultured on MS medium with half-strength of mineral salts. Flowering *in vitro* was also described in our experiments with *Drosera spathulata* and *Drosera rotundifolia* (Blehová *et al.* 1990) and was found to be seasonally dependent, expressing a maximum in April and May. It was connected with lower production of fresh and dry mass *in vitro*. Similar results were reported by Lowrie (1989) during the flowering period of *Drosera pygmaea* and other Australian sundew species growing under natural environmental conditions.

Gemma production *in vitro* and their germination described in this paper can be used for further structural and functional investigations. To obtain a more complex view of gemma production it is necessary to perform further cytological studies of gemma origin and development within the stipule bud formed *in vivo* or *in vitro*. We believe that plant reproduction using gemmae might represent an useful model system to study plant morphogenesis from a somatic cell (including *in vitro* experiments), and can provide an alternative to the widely used *in vitro* somatic embryogenesis.

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